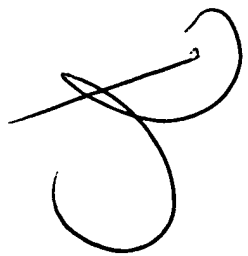


aeration and dispersal of air such that the L-lactate concentration of the product solution remains under 40 mmoles/l.

5,200,327

EXPRESSION SYSTEM FOR THE SECRETION OF BIOACTIVE HUMAN GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) AND OTHER HETEROLOGOUS PROTEINS FROM STREPTOMYCES

Robert T. Garvin, Toronto, and Lawrence T. Malek, Brampton, both of Canada, assignors to Cangene Corporation, Mississauga, Canada

Continuation-in-part of Ser. No. 795,331, Nov. 6, 1985, abandoned, and a continuation-in-part of Ser. No. 221,346, Jul. 18, 1988, abandoned. This application Jul. 26, 1988, Ser. No. 224,568

Claims priority, application Canada, Jul. 25, 1988, 572956

Int. Cl.[5] C12P *21/02*; C12N *15/76*, *15/00*, *1/21*

U.S. Cl. 435—69.5 21 Claims

1. A gene expression system comprising a regulatory nucleotide sequence operably linked to a nucleotide sequence encoding a heterologous protein, wherein

said regulator nucleotide sequence comprises a promoter sequence operably linked to a nucleotide sequence encoding a signal peptide;

said signal peptide is capable of directing the secretion of said heterologous protein in bioactive form from a host selected from the genus Streptomyces; and

said signal peptide is a hybrid of signal peptides of the genus Streptomyces.

7. A vector capable of transformation and replication in Streptomyces wherein said vector comprises a gene expression system of claim 1 or claim 5.

14. A process of producing a heterologous protein in a bioactive form that is secreted from a host selected from the genus Streptomyces comprising the steps of:

(A) transforming a host selected from the genus Streptomyces with a vector according to claim 7;

(B) growing a culture of the host produced by transformation with said vector under conditions such that said heterologous protein is expressed and secreted in said bioactive form; and

(C) recovering said heterologous protein from said culture.

5,200,328

PROCESS FOR PRODUCING METHYL GLYCOSIDE ESTERS

Ole Kirk, Copenhagen; Sven Erik Godtfredsen, Vaerlose, both of Denmark, and Fredrik Björkling, Helsingborg, Sweden, assignors to Novo Nordisk A/S, Bagsvaerd, Denmark

Filed Mar. 16, 1990, Ser. No. 494,702

Claims priority, application Denmark, Feb. 17, 1989, 0768/89

The portion of the term of this patent subsequent to Mar. 2, 2010, has been disclaimed.

Int. Cl.[5] C12P *19/04*

U.S. Cl. 435—101 11 Claims

1. A process for preparing a compound of formula I

$$R—COO—X—OCH_3 \quad (I)$$

wherein

R is alkyl with 7-24 carbon atoms optionally substituted by hydroxy or halogen; and

X is a monosaccharide containing one hexose or pentose unit which carries (a) the $—OCH_3$ group at the anomeric carbon atom and (b) the R—COO—group at a primary hydroxy group; comprising reacting (a) an acid or ester of formula II

$$R—COOR^1 \quad (II)$$

wherein

R is as defined above; and

R^1 is H or lower alkyl; with (b) a glycoside of formula III

$$X—OCH_3 \quad (III)$$

wherein

X is as defined above; in a substantially non-aqueous medium, in the substantial absence of a solvent other than the acid or ester of formula II acting as a solvent for the glycoside of formula III, and in the presence of an immobilized lipase.

5,200,329

METHOD OF HYDROXYLATING 3-[(4,7-DICHLOROBENZOXAZOL-2-YL)METHYL-]AMINO-5-ETHYL-6-METHYL-2-(1H)-PYRIDINONE BY INCUBATION WITH LIVER SLICES

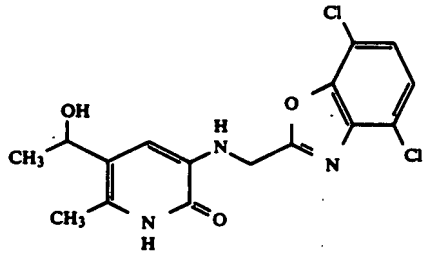
Suresh K. Balani, Hatfield; Anthony D. Theoharides, Lansdale, and Laura R. Kauffman, Jeffersonville, all of Pa., assignors to Merck & Co., Inc., Rahway, N.J.

Continuation of Ser. No. 771,963, Oct. 4, 1991, abandoned. This application Mar. 10, 1992, Ser. No. 850,008

Int. Cl.[5] C12P *17/16*

U.S. Cl. 435—118 2 Claims

1. A method of preparing the compound



or a pharmaceutically acceptable ester thereof, comprising the steps of

(1) providing a quantity of 3-[(4,7-dichlorobenzoxazol-2-yl)methyl]amino-5-ethyl-6-methyl-2-(1H)pyridinone,

(2) incubating the compound of step 1 with rat liver slices, and

(3) isolating the compound.

5,200,330

METHOD FOR THE PREPARATION OF METHYL ANTHRANILATE

Gregory V. Page, Maplewood; Bonita Scire, East Brunswick, and Mohamed I. Farbood, Holmdel, all of N.J., assignors to BASF K&F Corporation, Parsippany, N.J.

Division of Ser. No. 70,062, Jul. 6, 1987, abandoned. This application Sep. 14, 1990, Ser. No. 582,829

Int. Cl.[5] C12P *13/00*, *13/02*, *7/62*

U.S. Cl. 435—128 8 Claims

1. A method for the production of methyl anthranilate comprising:

providing a microorganism selected from the group consisting of *Trametes versicolor* ATCC 42394 and Polyporus sp. ATCC 10089;

incubating said microorganism under aerobic conditions with substrate methyl N-methyl anthranilate in a nutrient broth for a period of time at a pH and at a temperature effective to allow said microorganism to convert said substrate to methyl anthranilate, wherein said pH is in a range of from about 3 to about 9, said temperature is in a range of from about 18° C. to about 33° C. and said incubation period is from 1 to 14 days; and

recovering the methyl anthranilate.

tially pure R(+)-phenylethanol in the presence of NADPH, and wherein said dehydrogenase, in substantially pure form,

(A) has an optimum pH of 7 for reduction of acetophenone and an optimum Ph of 8 for oxidation of phenylethanol;

(B) has an optimum temperature of 25°-30° C.;

(C) has a K_M value of 6×10^{-4} M for acetophenone;

(D) has a K_M value of 1.4×10^{-4} M for NADPH; and

(E) is rapidly inactivated by EDTA but is only weakly inhibited by inhibitors and chelators selected from the group consisting of 2,2'-dipyridine, 1,10-phenanthroline, iodoacetamide, p-hydroxymercurybenzoate, N-ethylmaleimide, phenylmethanesulfonyl fluoride and Triton X-100 and SH-protecting reagents selected from the group consisting of dithiothreitol and glutathione.

5,200,336

RESTRICTION ENDONUCLEASE OBTAINABLE FOAM BACILLUS COAGULANS AND A PROCESS FOR PRODUCING THE SAME

Huimin Kong, Beverly, and Ira Schildkraut, Hamilton, both of Mass., assignors to New England Biolabs, Inc., Beverly, Mass.

Filed Jul. 2, 1990, Ser. No. 547,787
Int. Cl.[5] C12N *9/22, 1/00*
U.S. Cl. 435—199 6 Claims

1. A restriction endonuclease obtainable from *Bacillus coagulans*, ATCC No. 55055, where said endonuclease recognizes the following base sequence in double-stranded deoxyribonucleic acid molecules:

5'... ↓ (N)10CGA(N)6TGC(N)12 ↓ ... 3'
3'... ↑ (N)12GCT(N)6ACG(N)10 ↑ ... 5'

and cleaves said deoxyribonucleic acid molecules at both ends of the recognition sequence as indicated by the arrows.

5,200,337

NOVEL TYPE II RESTRICTION ENDONUCLEASE, APO I, OBTAINABLE FROM ARTHROBACTER PROTOPHORMIAE AND A PROCESS FOR PRODUCING THE SAME

Carol Polisson, Arlington; Derek Robinson, Boxford, and Keith Lunnen, Newbury, all of Mass., assignors to New England Biolabs, Inc., Beverly, Mass.

Filed Oct. 25, 1991, Ser. No. 782,515
Int. Cl.[5] C12N *9/22*
U.S. Cl. 435—199 12 Claims

1. A substantially pure Type II restriction endonuclease ApO I obtainable from *Arthrobacter protophormiae* (ATCC#55228) which recognizes and cleaves all permutations of the following base sequence in double-standard deoxyribonucleic acid molecules:

↓
5'-Pu A A T T Py-3'
3'-Py T T A A Pu -5'
↑

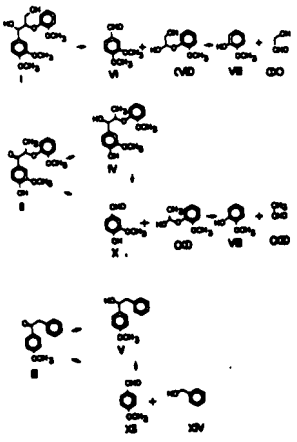
and having a cleavage defined by the arrows.

5,200,338

BACTERIAL EXTRACELLULAR LIGNIN PEROXIDASE

Donald L. Crawford, and Muralidhara Ramachandra, both of Moscow, Id., assignors to Idaho Research Foundation, Incorporation, Moscow, Id.

Filed Nov. 30, 1988, Ser. No. 277,802
Int. Cl.[5] C12N *9/24*
U.S. Cl. 435—200 16 Claims



1. A lignin peroxidase enzyme having a molecular weight of about 18 kD, enzyme activity that produces Cα-oxidation and Cα–Cβ cleavage of lignin and lignin degradation products, and a specific lignin peroxidase activity of greater than 0.30 enzyme U/mg.

5,200,339

PROTEASES CAUSING ABNORMAL DEGRADATION OF AMYLOID β-PROTEIN PRECURSOR

Carmela R. Abraham, 5 Blodgett Rd., Lexington, Mass. 02173

Continuation-in-part of Ser. No. 568,806, Aug. 17, 1990, abandoned. This application Apr. 5, 1991, Ser. No. 681,093
Int. Cl.[5] C12N *9/48;* C12Q *1/37;* A61K *37/547*
U.S. Cl. 435—212 2 Claims

1. A brain-derived, calcium activated proteolytic factor comprising a serine protease that cleaves β-protein precursor at a site outside the β-protein domain and near the β-protein N-terminus, said serine protease migrating as a band having a molecular weight of about 30 kDa and a band having a molecular weight of about 68 kDa, as estimated by 12% SDS-PAGE using as molecular weight standards phosphorylase B, bovine serum albumin, ovalbumin and carbonic anhydrase.

5,200,340

THROMBIN-ACTIVATED TISSUE PLASMINOGEN ACTIVATORS

Donald C. Foster; Eileen R. Mulvihill; Patrick J. O'Hara, all of Seattle, Wash.; Kurt Pingel, Farum, Denmark, and Shinji Yoshitake, Ibaraki, Japan, assignors to ZymoGenetics, Inc., Seattle, Wash.

Filed May 22, 1987, Ser. No. 53,412
Int. Cl.[5] C12N *9/61, 15/00, 15/58, 15/35*
U.S. Cl. 424—94.64 11 Claims

1. A single chain form of a human t-PA, wherein said single chain form is cleavable by thrombin, said cleavage resulting in stimulation of amidolytic activity.

10) K-value:
the K-value for the substrate N-acetyl-2,3-didehydroleucine is 4.5 mM (30° C., 0.1M glycine buffer, pH 9).

5,212,070

SECRETORY SIGNAL SELECTION VECTORS FOR EXTRACELLULAR PROTEIN SYNTHESIS IN BACILLI

Hilde E. Smith, Groningen; Jan H. Van Ee, Nieuwerkerk a/d IJssel; Ben P. H. Peeters, Haren; Sierd Bron, Haren, and Gerard Venema, Haren, Netherlands, assignors to Gist-brocades, Netherlands

Continuation of Ser. No. 45,890, May 1, 1987, Pat. No. 5,037,760. This application Dec. 13, 1990, Ser. No. 627,028
Int. Cl.[5] C12P *21/02, 19/34;* C12N *15/00, 1/21;* C07H *15/12;* A61K *37/02*
U.S. Cl. 435—69.1 4 Claims

1. In a method for producing a peptide product by recombinant techniques, wherein a host microorganism is transformed with a plasmid comprising a gene encoding said peptide product, and growing said host under nutrient conditions, whereby said gene is expressed and said peptide product is secreted, the improvement which comprises:

employing as said gene an open reading frame encoding said peptide product joined at its 5' terminus to a DNA sequence encoding an amino acid sequence capable of functioning as a secretory signal sequence (hereinafter "secretory sequence"), wherein said open reading frame DNA sequence is other than the native open reading frame of said DNA sequence encoding said secretory sequence and wherein said secretory sequence comprises one of the following amino acid sequences.

Met arg lys ser leu ile thr leu gly leu ala ser val
ile gly thr ser ser phe leu ile pro phe thr ser lys
thr ala ser ala glu thr leu asp glu lys lys gln lys
ile glu ser lys gln ser glu val ala ser ser ile glu
ala lys glu lys glu leu thr glu;

Met lys lys met leu val val leu leu phe ser ala leu leu
leu asn gly cys gly ser gly glu ser lys ala asn thr ala
glu thr pro glu val leu asp val lys leu thr gly;

Met ile arg gly ile leu ile ala val leu gly ile ala ile
val gly;

Met leu lys lys val ile leu ala ala phe ile leu val gly
ser;

Met ser gln gln his asp tyr val ile gly lys asn ala val
ile glu thr leu lys ser asp arg leu asp leu phe pro leu
leu arg leu thr lys lys pro lys val gln thr gly ile asp
thr leu leu pro asp tyr lys lys gln;

glu phe glu leu ala pro gly leu phe ile leu leu phe leu
phe val met ala val ile gly;

Met leu lys arg thr ser phe val ser ser leu phe ile ser
ser ala val leu leu ser ile leu leu pro ser gly leu ser
his thr leu ser ala lys gly thr asn lys asn asn leu phe
phe phe asp thr glu thr thr gly leu gly gly gly ala gly
asn thr ile phe leu leu gly his ala arg val tyr glu asp
arg val thr val lys gln his leu leu pro lys pro lys asn
glu val ala leu tyr gln ser phe leu ser glu val asp ile
thr ser leu val thr tyr asn gly lys ala phe asp tyr;

Met lys ile ser arg ile leu leu ala ala val ile leu ser
ser val phe ser ile thr tyr leu gln ser asp leu gly trp
phe ala lys glu gly gln met asp glu thr phe thr lys ala
ala phe lys leu lys thr gly glu val ser asp;

Met lys gln thr val leu leu leu phe thr ala leu phe leu
ser gly cys ser val ala ser ala asp asp ser val pro arg
phe thr glu glu gly lys tyr ile gly ser ala asp;

Met lys lys leu val phe gly leu leu ala ile val leu phe
gly cys gly leu tyr ile tyr his val trp phe gly asp;

-continued

Met leu lys lys cys ile leu leu val phe leu cys val gly
leu ile gly leu ile gly cys ser lys thr asp ser pro glu
asp;

Met arg lys trp ile ala ala ala gly leu ala tyr val leu
tyr gly leu phe phe tyr trp tyr phe phe leu ser gly asp
set ala ile pro glu ala val lys gly thr gln ala asp;

Met pro ile lys lys lys val met met cys leu ala val thr
leu val phe gly ser met ser phe pro thr leu thr asn ser
gly gly phe lys glu ser thr asp; and

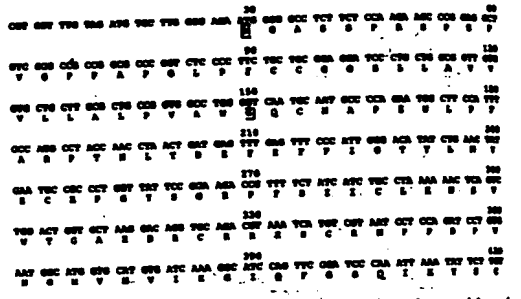
Met lys leu val pro arg phe arg lys gln trp phe ala tyr
leu thr val leu cys leu ala leu ala ala ala val ser phe
gly val pro ala lys ala ala glu asn pro gln thr ser val
ser asn thr gly lys glu ala asp ala thr lys asn gln thr
ser lys ala asp.

5,212,071

NUCLEIC ACIDS ENCODING A HUMAN C3B/C4B RECEPTOR (CR1)

Douglas T. Fearon, Baltimore, Md.; Lloyd B. Klickstein, Brookline, Mass.; Winnie W. Wong, Wahan, Mass.; Gerald R. Carson, Wellesley, Mass.; Michael F. Concino, Newton, Mass.; Stephen H. Ip, Sudbury, Mass., and Savvas C. Makrides, Bedford, Mass., assignors to The Johns Hopkins University, Baltimore, Md.; Brigham and Women's Hospital, Boston and T Cell Sciences, Inc., Cambridge, both of Mass.

Continuation-in-part of Ser. No. 176,532, Apr. 1, 1988, abandoned. This application Apr. 3, 1989, Ser. No. 332,865
Int. Cl.[5] C12N *15/12, 15/63*
U.S. Cl. 435—69.1 64 Claims



1. An isolated nucleic acid encoding a polypeptide, the amino acid sequence of which comprises at least a fragment of the amino acid sequence depicted in FIG. 1, which polypeptide has a complement regulatory activity.

5,212,072

POLYPEPTIDES COMPLEMENTARY TO PEPTIDES OR PROTEINS HAVING AN AMINO ACID SEQUENCE OR NUCLEOTIDE CODING SEQUENCE AT LEAST PARTIALLY KNOWN AND METHODS OF DESIGN THEREFOR

J. Edwin Blalock; Kenneth L. Bost, both of Birmingham, Ala., and Eric M. Smith, Galveston, Tex., assignors to Board of Regents, The University of Texas System, Austin, Tex.

Continuation of Ser. No. 829,709, Feb. 19, 1986, which is a continuation-in-part of Ser. No. 708,001, Mar. 1, 1985, Pat. No. 4,863,857. This application Mar. 7, 1991, Ser. No. 665,967
Int. Cl.[5] C12P *21/06*
U.S. Cl. 435—69.1 23 Claims

1. A polypeptide complementary to at least a portion of an original peptide or protein, said polypeptide being produced by a process comprising the steps of:

(a) determining a first nucleotide sequence of a first nucleic acid, said first nucleotide sequence coding for an amino